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THE CLINICAL USE OF CORD BLOOD-DERIVED VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES REACTIVE AGAINST CYTOMEGALOVIRUS (CMV), ADENOVIRUS, AND EPSTEIN-BARR VIRUS (EBV)

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CMV, Adenovirus (Ad) and EBV are viral pathogens causing morbidity and mortality in patients after hematopoietic stem cell transplantation (HSCT) and cord blood transplantation (CBT). We have shown that adoptive immunotherapy with peripheral blood donor-derived multivirus-specific Cytotoxic T Lymphocytes (mCTL) directed against EBV, CMV and Ad can effectively prevent and treat the clinical manifestations of these viruses after HSCT. We have now extended these studies by expanding mCTL from umbilical cord blood (CB) to restore cellular immunity to CMV, EBV and Ad simultaneously after CBT. However, the development of mCTL for patients undergoing CBT requires the priming and extensive expansion of naïve T cells rather than the more limited and simple direct expansion of pre-existing memory T cell populations from virus-experienced donors. We have developed a novel protocol utilizing an initial round of stimulation with autologous CB-derived dendritic cells transduced with a recombinant Ad5f35 vector carrying a transgene for the immunodominant CMV antigen, pp65 (Ad5f35pp65) in the presence of IL12, IL15 and IL7. This is followed by 2 rounds of weekly stimulation with autologous EBV-LCL transduced with the same vector in the presence of IL15 and IL2. After 3 rounds of stimulation, 2 CTL cultures generated for clinical use contained a mean of 48% (range 24-72%) CD8+, and 42% (range 11-72%) CD4+ cells with mean 30.5% (range 23-46%) CD45RA-/CD62L+ T cells. In ^{51}Cr release and/or IFN-γ ELISPOT assays, both mCTL lines showed specific activity against CMV, EBV and Ad targets. So far we have treated 2 patients in this phase I study, both on dose level 1 ($5x10^6/m^2$). Patients received 1 infusion of CTL on day 63 post-CBT. Only patient 1 is currently evaluable as patient 2 was infused too recently to test. We observed a 10-fold increase in CMV-specific T cells 5 weeks-post CTL. This patient was transiently positive for CMV by PCR at 4 weeks post CTL but was negative within 16 days of receiving a second dose of mCTL with a corresponding rise in CMV-specific CTL detected in the peripheral blood. The patient became Ag+ for Ad in his stool associated with diarrhea which resolved spontaneously without additional therapy. In summary, we have developed a protocol for the generation of mCTL from CB: infusion of small numbers of these cells increased virus-specific T cells in the peripheral blood post CTL infusion dramatically earlier than observed immune reconstitution post CBT.

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DRAMATIC REGRESSION OF CHRONIC LYMPHOCYTIC LEUKEMIA IN THE FIRST PATIENT TREATED WITH DONOR-DERIVED GENETICALLY-ENGINEERED ANTI-CD19-CHIMERIC-ANTIGEN-RECEPTOR-EXPRESSING T CELLS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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We have initiated a clinical trial in which patients receive infusions of allogeneic T cells that are genetically modified with a gammaretroviral vector to express a chimeric antigen receptor (CAR) that recognizes the B-cell antigen CD19. The first patient treated on this trial was a 65 year-old man with chronic lymphocytic leukemia (CLL) who relapsed after HLA-matched unrelated donor hematopoietic stem cell transplantation. Following the relapse, the patient received 4 donor lymphocyte infusions (DLIs) with a maximum $CD3^+$ cell dose of $2.9x10^7$/kg and then a second stem cell transplant from the original donor. An objective remission of the leukemia did not occur after any of the DLIs or the second transplant. Five months after the second transplant, when his CLL was progressing, the patient received an infusion of $6.2x10^7$ ($1x10^6$ cells/kg) allogeneic anti-CD19-CAR-transduced T cells derived from his unrelated transplant donor. Thirty-nine percent of the infused cells expressed the anti-CD19 CAR, and the cells produced interferonγ and IL-2 in a CD19-specific manner. The patient did not receive any other therapy in conjunction with the CAR-transduced T cells. From 6 to 12 days after the CAR-transduced T cell infusion, the patient experienced fevers, fatigue, mild hypoxemia, and intermittent mild hypotension. Increases in serum magnesium, phosphorous, and uric acid consistent with tumor lysis syndrome occurred. A decrease in cardiac left ventricular function developed, which was improving at last follow-up. The patient's blood B cell count decreased from 286 cells/μL before the CAR-transduced T cell infusion to 0 cells/μL 26 days after the cell infusion. Before the CAR-transduced T cell infusion, CLL cells made up 80-90% of the patient's hypercellular bone marrow. A bone marrow biopsy performed 26 days after the cell infusion showed a normocellular marrow, nearly absent B-lineage cells, and no evidence of CLL. CT scans revealed a greater than 50% decrease in the size of multiple lymph nodes after the CAR-transduced T cell infusion, but residual adenopathy was present. CAR-transduced cells were not detected in the patient's blood by quantitative PCR during the first week after the T cell infusion, but made up 0.98% of blood mononuclear cells 11 days after the infusion. These results are encouraging for further development of anti-CD19-CAR-expressing T cells as a treatment for relapse after allogeneic stem cell transplantation.

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ALLOGENEIC VIRUS-SPECIFIC T CELLS WITH HLA ALLOREACTIVITY DO NOT PRODUCE GRAFT-VERSUS-HOST DISEASE IN HUMAN SUBJECTS

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We and others have recently established that T cell reactivity with non-self HLA (HLA alloreactivity) arises not only from naive T cells but also from the antigen-experienced T cell pool, including Epstein-Barr virus (EBV) and cytomegalovirus (CMV)-specific T cells. Virus-specific T cells could therefore mediate graft-versus-host disease (GvHD) if infused into partially HLA mismatched recipients. We reviewed our clinical experience with adoptive transfer of allogeneic hematopoietic stem cell transplant donor-derived virus-specific T cell lines in 153 recipients, including 73 partially HLA-mismatched recipients. The degree of HLA mismatching varied from one allele to a full haplotype. *De novo* GvHD did not develop after infusion of cytotoxic T lymphocytes (CTL), and the incidence of GvHD reactivation was 6.5% and not significantly different between recipients of HLA matched or mismatched CTL. Thus, virus-specific CTL did not mediate GvHD, even in recipients of partially matched CTL. Next we analyzed the HLA alloreactivity of four donor-infused bivirus-specific T cell lines, using activated T cells, that are known to lack CMV and EBV antigen expression, as antigen presenting cells (TAPC). We used a panel of 44 TAPC covering the most frequent HLA class I and II alleles. The CTL lines were labeled with CFSE and stimulated with TAPC for 6 hours, after which production of TNFα and IFNγ/IL-2 by CD4+ and CD8+ T cells in the CFSE-positive fraction was analyzed by flow cytometry. All CTLs responded to a number of TAPC, with some APC being recognized strongly. The majority elicited only weak or no response from the CTLs. We then assessed whether the CTLs recognized TAPC expressing the recipient's HLA alleles. We found moderate reactivity of the CTL with 1-5 TAPC expressing recipient HLA alleles. Taken together, our data indicate that reactivity of virus-specific CTLs with hematopoietic APC does not correlate with the risk of developing GvHD, and that virus-specific CTL can safely be infused into HLA class I and/or II mismatched recipients.

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PHASE I CLINICAL TRIAL TARGETING CD20+ NON-HODGKIN'S LYMPHOMA (NHL) AFTER AUTOLOGOUS STEM CELL TRANSPLANT WITH ANTI-CD3 × ANTI-CD20 BISPECIFIC ANTIBODY ARMED T CELLS

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Relapse rates in patients (pts) after autologous stem cell transplant (SCT) for high risk or refractory NHL remains high. Our preclinical studies showed that activated T cells (ATC) armed with anti-CD3 x anti-CD20 bispecific antibody (CD20Bi) lyse rituximab-resistant CD20+ lymphoma cells. A phase I trial was conducted in 15 high risk or refractory NHL pts to determine the safety of armed ATC (aATC) given after SCT and whether aATC infusions accelerate immune recovery and provide an anti-lymphoma effect. Leukopheresis products were activated with anti-CD3, expanded in IL-2, armed with CD20Bi, and cryopreserved for infusions after SCT. At the time of SCT, there were 6 pts in 1st or 2nd remission (CR), 4 pts had refractory disease (PRD), 4 pts were in partial response, and 1 pt had progressive disease (PD). Pts received 5-20 $\times$ 10^9 aATC per infusion. The first 3 patients received $5x10^9$ three times per week for 3 weeks and then once per week for 6 weeks for a total aATC dose of $75x10^9$. Subsequently, the schedule was revised to 1 infusion/week for 4 weeks with infusion doses of 10, 15, and $20x10^9$ aATC. The median dose of CD34+ cells/kg infused was $4.0x10^6$(1.04-12.3x10^6). The ATC product was 92% viable, contained medians of 96.5% CD3, 67% CD4, and 48.9% CD8 cells. Fifteen pts received aATC and 12 were evaluable for toxicities. The Table 1 summarizes grade 1-3 events.

Table 1. Grade-1-3 Events

Dose	5 billion	10 billion	15 billion	20 billion
Pts Number	3	3	3	3
Infusions	15	4	4	4
Fever	3	10	12	12
Chills	2	7	3	4
Malaise	1	2	7	11
Hypotension	3	4	1	5
Tachycardia	3	2	5	4
Nause/vomiting	0	7	3	4
Headache	0	2	2	2
Dyspnea	1	0	0	0
Hypoxia	0	0	0	1

(NCI Immunotherapy Criteria).

The median day to engraftment was 14.75 days without G-CSF. aATC could be detected by flow cytometry up to 12 hours after infusion. The proportions of CD4 cells were in the normal range up to a month. There was a $>$ 4x increase in the mean ($\pm$SD) number of IFNγ EliSpots in response to Daudi cells from $30.5\pm20.5/10^6$ PBL preSCT to $125.6\pm130/10^6$PBL post SCT, $p < 0.008$. The median cytotoxicity mediated by NK cells ranged from 8.2-13.7% between 2 weeks and 3 months after SCT. Serum cytokines and chemokines peaked around 4 hrs after aATC infusions with 1-2 log increases in IL-2, IL-7, IL-15, IL-2r, MIP-1β, IP10, MIP-1α, MIG, and MICP-10. At 90 days after SCT, 9 pts were in CR and 6 pts had PD. The median OS has not been reached and OS is projected to be 55% at 4 years. Pts in CR prior to SCT had longer survival at 85% with no relapses after 1 year after SCT. These findings show that: 1) ATC can be expanded from heavily pretreated NHL pts; 2) aATC infusions are safe; 3) induce high levels of serum cytokines and chemokines; and 4) may provide anti-tumor help and cytotoxicity after SCT.

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COMBINING AZACITIDINE (VIDAZA®, AzA) AND DONOR LYMPHOCYTE INFUSIONS (DLI) AS FIRST SALVAGE TREATMENT IN PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML) OR MYELODYSPLASTIC SYNDROMES (MDS) RELAPSING AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (Allo-SCT): INTERIM-ANALYSIS FROM THE AZARELA-TRIAL (NCT-00795548)

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Prognosis for pts with AML/MDS relapsing after allo-SCT is poor and therapeutic options are limited. Results from recent retrospective analyses on the use of Aza +/- DLI in pts with AML/MDS, who relapsed after allo-SCT, were encouraging.

To evaluate the potential of Aza combined with DLI as 1st salvage therapy in pts with AML/MDS relapsing after allo-SCT we conducted a prospective phase-II multicenter trial. Pts were allowed to receive up to 8 cycles Aza (100 mg/m^2/d d1-5, every 28 d) and 3 DLI with increasing dosages ($1-5x10^6$–$1-5x10^8$ cells/kg) after every 2nd Aza cycle. So far, 25 pts (15 f/10 m) are evaluable for this analysis.

At diagnosis 23 (92%) had AML (15 de novo/8 following MDS), 1 (4%) MDS (RAEB-1) and 1 (4%) MDS/MPS (CMML-1). Based on cytogenetics 21 pts belonged to an adverse or intermediate risk group, whereas 2 pts had favorable cytogenetics (2 n.p.).

At transplant, 6 pts (24%) had induction failure, 6 (24%) suffered from 1st or 2nd relapse, 10 pts (40%) were in 1st or 2ndCR, while 3 pts (12%) were untreated. Eight pts (35%) received grafts from MSD and 15 (65%) from MUD (2 pts missing data). PBSC were used in 24 pts (96%; 1 missing). Prior to relapse 9 (36%) and 3 (12%) pts had episodes of aGvHD and/or cGvHD, respectively.

At relapse, 4 (31%) of 13 evaluable pts had normal cytogenetics, while 9 (69%) had chromosomal aberrations including 6 pts (46%) with complex karyotype. Relapse occurred in median 160 d (range 19-1199) after allo-SCT (BM blasts 34%, range 5-100%, donor chimerism 63% range 1-100%). Median age was 54 y (range 29-71). Patients received a median of 3 cycles Aza (range 1-8) and 18 pts (72%) received DLI (median: 1, range 1-4, median CD3 dose $5x10^6$/kg/DLI, range $1-207x10^6$).

Overall response rate was 64% with 5 pts (20%) achieving CR/CRi, 3 (12%) PR and 8 (32%) SD. Median response duration was 266 d. Acute GvHD occurred in 6 pts (24%) (2 skin/6 liver/ 2 gut) after a median of 65 d (range 19-179) following the 1st DLI. Three pts (12%) had limited cGvHD. Hematotoxicity III°-IV° was observed in 64%. Common adverse events were gastrointestinal side effects and infections. After a median follow-up of 100 d (range 25-485) 15 pts (60%) are alive. Median OS of all pts is 184 d (range 87-281). All pts, who achieved a CR/CRi, remained in ongoing remission for a median of 229 d.

Our data suggest that salvage therapy with Aza + DLI for pts with AML/MDS relapsing after allo-SCT is feasible and has significant anti-leukemic activity in these pts.

GRAFT PROCESSING

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A COST EFFECTIVE ANALYSIS OF A RISK-ADAPTED ALGORITHM FOR PLERIXAFOR USE IN AUTOLOGOUS PERIPHERAL BLOOD STEM CELL MOBILIZATION

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Up to 30% of patients fail to collect minimum numbers of PBSC for ASCT, and up to 50% of patients fail to collect the optimal number. Plerixafor, a CXCR4 antagonist, in combination with G-CSF has shown superior results in mobilizing PB CD34+ cells in comparison to G-CSF alone for autologous PBSC mobilization in patients with NHL or MM. However, due to its high cost, we commenced a risk adapted algorithm for the utilization of plerixafor starting in Feb 2009.

Jan-Dec 2008 was the baseline. Patients in upfront mobilization clinical trials with plerixafor were excluded. From Feb-Nov 2009, the risk adapted algorithm Plerixafor-1 was used. PBSC mobilization was commenced with G-CSF at 10 mcg/kg/day. If PB CD34 on day 4 or day 5 was $\geq$ 10/μL, apheresis was commenced the next morning.